Plant Pathology (2004) **53**, 759–769 Doi: 10.1111/j.1365-3059.2004.01103.x

Characterization of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*

W. Chen[a]*†, C. J. Coyne[b], T. L. Peever[c] and F. J. Muehlbauer[a]

[a]*USDA – ARS, Grain Legume Genetics and Physiology Research Unit;* [b]*USDA – ARS, Western Regional Plant Introduction Station; and* [c]*Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA*

Forty-eight chickpea germplasm lines, including 22 differentials used in previous studies, were characterized for disease phenotypes following inoculation with six isolates of *Didymella* (anamorph *Ascochyta*) *rabiei*, representing a wide spectrum of pathogenic variation. Representative isolates were also directly compared with six previously identified races on eight chickpea genotypes. Many of the chickpea differentials reacted similarly to inoculation with each isolate of *D. rabiei*, and several previously identified races caused similar levels of disease on the differentials. This indicates that the number of differentials can be reduced significantly without sacrificing accuracy in describing pathogenic variation of *D. rabiei* on chickpea. Pathogenic variation among samples of US isolates allowed classification of the isolates into two pathotypes. The distribution of disease phenotypes of the 48 germplasm lines was bimodal after inoculation with pathotype I isolates, whereas the distribution of disease phenotypes was continuous after inoculation with pathotype II isolates. Such distinct distribution patterns suggest that chickpea plants employ different resistance mechanisms to each pathotype and that the two pathotypes may have different genetic mechanisms controlling pathogenicity. The advantages of using the two-pathotype system in assaying pathogenicity of the pathogen and in studying resistance mechanisms of the host are discussed. Three chickpea accessions, PI 559361, PI 559363 and W6 22589, showed a high level of resistance to both pathotypes, and can be employed as resistance sources in chickpea breeding programmes for resistance to ascochyta blight.

Keywords: ascochyta blight, *Ascochyta rabiei*, chickpea, differentials, pathogenicity assay, pathotypes, resistance

Introduction

Ascochyta blight caused by *Didymella* (anamorph *Ascochyta*) *rabiei* is an important disease of chickpea worldwide, and has been a persistent and major disease problem in the US Pacific Northwest. The disease affects all aboveground parts of the plant and can lead to total destruction of the crop. Severe epidemics of ascochyta blight have occurred many times in various production regions, often on cultivars previously thought to be resistant (Nene, 1982; Singh *et al*., 1984; Nene & Reddy, 1987). The evolution of a new race or virulence form is frequently invoked to explain such outbreaks (Reddy & Kabbabeh, 1985; Porta-Puglia *et al*., 1996). Various terms such as pathogenic groups, races, virulence forms and pathotypes have been proposed as a means to classify the pathogenic variation of *D. rabiei* isolates. Vir & Grewal (1974) described 13 pathogenic groups in India. Reddy & Kabbabeh (1985) reported six races of *D. rabiei* from Syria and Lebanon using six chickpea differentials. Singh (1990) reported 11 races among 13 isolates, and Jan & Wiese (1991) identified 11 virulence forms among 39 isolates of *D. rabiei* in the Palouse region of the US using 14 differential chickpea lines. Navas-Cortés *et al*. (1998) identified 11 pathotypes in 44 isolates from India, Pakistan, Spain and the United States using seven differentials. Udupa *et al*. (1998) used a three-pathotype system based on pathogenicity on three differentials. More recently, Chongo *et al*. (2004) grouped 40 Canadian isolates into 14 pathotypes based on pathogenicity on eight chickpea differentials.

Two main factors have contributed to such a profusion of classification schemes for pathogenic variation in *D. rabiei*. First, researchers have used different sets of differentials and different sets of isolates. Secondly, there is no standard method for scoring disease severity and no standard criteria for defining resistance and susceptibility. There are several methods for scoring ascochyta blight phenotype (Vir & Grewal, 1974; Gowen *et al*., 1989; Riahi *et al*., 1990; Chongo & Gossen, 2001; Lichtenzveig

*To whom correspondence should be addressed.

†E-mail: w-chen@wsu.edu

Accepted 23 July 2004

et al., 2002). The nonparametric 1–9 rating scale of Reddy & Singh (1984) is the most commonly used method, but it has been employed in different ways by different researchers, as discussed by Lichtenzveig *et al*. (2002). The various studies often used arbitrary criteria in defining resistance and susceptibility, and sometimes only categorical data (either resistant or susceptible) were presented (Reddy & Kabbabeh, 1985; Jan & Wiese, 1991) without accompanying quantitative disease scores. When both actual disease scores and categorical data are presented, many inconsistencies can be found between the disease scores and categorical classification within a single study (Chongo *et al*., 2004). These inconsistencies have made it difficult to compare results from one study to another.

Didymella rabiei is a heterothallic ascomycete with two mating types, and both mating types are present in equal frequency in most chickpea production regions (Trapero-Casas & Kaiser, 1992a; Kaiser & Kusmenoglu, 1997; Navas-Cortés *et al*., 1998; Barve *et al*., 2003). In addition, multilocus genetic disequilibrium tests using a set of unlinked molecular markers indicated that the fungus is randomly mating in the US Pacific Northwest (Peever *et al*., 2004) and probably in other areas as well. Pseudothecia and ascospores of *D. rabiei* usually develop on chickpea debris during winter months under cold and moist conditions, and play an important role as primary inoculum at the beginning of each growing season (Trapero-Casas & Kaiser, 1992b; Trapero-Casas *et al*., 1996). Genetic recombination contributes to increased levels of genotypic diversity of the pathogen (Geistlinger *et al*., 1997; Jamil *et al*., 2000; Santra *et al*., 2001; Peever *et al*., 2004), and is likely to result in a complete lack of correlation between neutral genetic markers and pathogenicity in natural populations (Navas-Cortés *et al*., 1998; Udupa *et al*., 1998; Jamil *et al*., 2000; Santra *et al*., 2001).

Planting resistant cultivars is the most economical approach to stabilizing and improving production of chickpea (Muehlbauer & Kaiser, 1994). Resistant cultivars like Dwelley and Sanford developed in the US provided effective control when they were first released in the early 1990s (Muehlbauer *et al*., 1998a,b). In recent years, increased incidence of ascochyta blight has been observed and this could be due to increased pathogenicity of the pathogen population or higher inoculum pressure. New chickpea cultivars with improved resistance to the current population of *D. rabiei* in the US are needed in order to maintain chickpea productivity. A reliable pathogenicity assay that will facilitate the screening process of breeding materials has recently been developed (Chen & Muehlbauer, 2003). However, in order to maintain an effective and efficient resistance breeding programme, there is a need to understand pathogenic variation in the pathogen population in the production area and to have access to resistance sources that provide better resistance than currently available in commercial cultivars (Porta-Puglia *et al*., 1994).

There are four objectives in this study: (i) to characterize pathogenic variation among isolates of *D. rabiei* from the western United States; (ii) to characterize chickpea germplasm lines that were previously used as differentials for pathogenicity assays of ascochyta blight; (iii) to compare US isolates with previously identified races of *D. rabiei*; and (iv) to identify chickpea accessions with better resistance than is currently available in commercial chickpea cultivars and advanced breeding lines in the US.

Materials and methods

Plant materials

A total of 48 chickpea accessions were used in this study (Table 1). They included 22 differentials that were previously used to measure pathogenicity of *D. rabiei* (Reddy & Kabbabeh, 1985; Jan & Wiese, 1991; Porta-Puglia *et al*., 1996; Navas-Cortés *et al*., 1998), seven germplasm lines that were reported as resistance sources (Reddy & Singh, 1992; Singh *et al*., 1997), and 19 commercial cultivars and advanced breeding lines.

Fungal isolates and maintenance

A total of 44 isolates of *D. rabiei* were used in this study (Table 2). These were either isolated from diseased plants collected from California, Idaho and Washington from 2000 to 2002, or obtained as pure cultures from the *D. rabiei* collection maintained at the USDA Western Regional Plant Introduction Station. To isolate the fungus from diseased plants, stems showing typical ascochyta blight symptoms were cut into 1 cm segments, surface-disinfested in 6% sodium hypochlorite for 3 min, rinsed in sterile distilled water for 3 min, and then blotted dry on sterile paper towels. Stem pieces were placed on either potato dextrose agar (PDA) or 2% water agar and incubated at 20°C for 7–10 days. Isolates were maintained either on cellulose filter paper or as conidia in sterile distilled water at 4°C. All isolates used in this study originated from single conidia (pycnidiospores).

Pathogenicity assay

The mini-dome technique previously described by Chen & Muehlbauer (2003) was used to measure pathogenic variation throughout this study except for the field evaluations. Chickpea seeds were planted in 6 × 25 cm Deepots (Stuewe & Sons, Inc., Corvallis, Oregon, USA) in either a growth chamber or glasshouse. Conidial suspensions were standardized by cultural age and spore concentration. Conidia of individual isolates were harvested from 2-week-old cultures on V-8 agar (200 mL V8 juice, 3 g $CaCO_3$ and 20 g Difco agar L^{-1}) by flooding pycnidial bearing colonies with sterile distilled water and dislodging spores with a sterile glass rod. Conidial concentrations were determined with a haemocytometer and adjusted to 2×10^5 pycnidiospores mL^{-1}. Two-week-old plants were sprayed with this conidial suspension to run-off (approximate 2 mL per plant) and immediately covered with an inverted translucent plastic cup to form a mini-dome to

Table 1 Chickpea germplasm lines used in this study

Germplasm accession[a]	Alternative identifier	Seed type[b]
B-90	NA[e]	SK
Billy Beans	NA	SK
Bronic	NA	SK
Burpee	NA	LK
Blanco Lechoso	NA	LK
CA9990I875 W	NA	LK
CA9890233W[c]	NA	LK
CA9990I604C[c]	NA	LK
Dwelley[cd]	PI 598079	LK
Evans[c]	PI 619100	LK
Myles[c]	PI 598080	D
Sanford[c]	PI 598078	LK
Sarah[c]	PI 543921	D
Sierra[c]	NA	LK
Spanish White[cd]	NA	LK
Surutato-77[c]	W6 17605	LK
UC 27[c]	PI 552530	LK
CDC-Yuma[c]	NA	SK
W6 2909	ILC 1272	LK
W6 22575	ILC 0072	SK
W6 22576	ILC 0182	SK
W6 22577	ILC 0191	SK
W6 22578	ILC 194	SK
W6 22579	ILC 200	SK
W6 22580	ILC 215	LK
W6 22581[d]	ILC 249	SK
W6 22582	ILC 482	SK
W6 22583	ILC 484	SK
W6 22584[d]	ILC (1929)	SK
W6 22585[d]	ILC 3279	SK
W6 22586	ILC 1591	D
W6 22587[d]	ICC (1903)	D
W6 22588	ILC 2232	D
W6 22589[d]	ICC 3996	D
W6 22590	ILC 4107	D
W6 22591	ILC 4935	D
W6 22592	ILC 5127	D
FLIP 84-92C	PI 614731	SK
PI 359075	RPIP12-069103	D
PI 559360	ILC 6482	SK
PI 559361	ICC 4475	D
PI 559362	ICC 6328	SK
PI 559363[d]	ICC 12004	D
PI 594328	FLIP 91-178C	SK
PI 594329	FLIP 93-53C	SK
PI 594330	FLIP 93-98C	SK
PI 552788	ILC 482	SK
PI 315818	ILC 249	SK

[a]All germplasm lines were used in differential characterization experiments, and are either from the USDA Western Regional Plant Introduction Station, Pullman, WA, or from the breeding programmes of the USDA Grain Legume Genetics and Physiology Research Unit, Pullman, WA.
[b]D, desi type; LK, large kabuli type; SK, small kabuli type.
[c]Germplasm lines used in field evaluation.
[d]Germplasm lines used in comparison of pathotypes with previously identified races.

Table 2 List of *Didymella rabiei* isolates used in this study

Isolate	Mating type[a]	Geographic location	Year of isolation
A2-11L[b]	nt[c]	Genesee, ID, USA	2002
A3-2S[b]	nt[c]	Genesee, ID, USA	2002
AR19 (ATCC 24891)[b]	2	Iran	1973
AR20 (ATCC 76501)	2	Genesee, ID, USA	1986
AR21 (ATCC 76502)[b]	1	Genesee, ID, USA	1986
AR169 (race 3)[d]	1	Syria	1996
AR628[b]	1	Syria	1995
AR650 (race 1)[d]	1	Syria	na[e]
AR651 (race 2)[d]	1	Syria	na[e]
AR652 (race 3)[d]	2	Syria	na[e]
AR653 (race 4)[d]	1	Syria	na[e]
AR654 (race 5)[d]	2	Syria	na[e]
AR655 (race 6)[d]	1	Syria	na[e]
AR737	1	Pullman, WA, USA	1996
CAB01-1	1	Pullman, WA, USA	2001
CAB01-2	1	Pullman, WA, USA	2001
CAB01-3	1	Pullman, WA, USA	2001
CAB01-4	1	Pullman, WA, USA	2001
CAB01-6	1	Pullman, WA, USA	2001
CAB01-7	1	Pullman, WA, USA	2001
CAB01-8	1	Pullman, WA, USA	2001
CAB02-1	1	Fresno, CA, USA	2002
CAB02-2	1	Fresno, CA, USA	2002
CAB02-3	1	Fresno, CA, USA	2002
CAB02-4	1	Fresno, CA, USA	2002
CAB02-5	1	Fresno, CA, USA	2002
CAB02-11	1	Sutter County, CA, USA	2002
CAB02-12	1	Sutter County, CA, USA	2002
CAB02-13	1	Sutter County, CA, USA	2002
CAB02-14[b]	1	Sutter County, CA, USA	2002
CAB02-15	1	Sutter County, CA, USA	2002
CAB02-16	1	Sutter County, CA, USA	2002
CAB02-17	1	Sutter County, CA, USA	2002
CAB02-18	1	Sutter County, CA	2002
CAB02-19	1	Sutter County, CA, USA	2002
CAB02-20	1	Sutter County, CA, USA	2002
EV00-22	2	Genesee, ID, USA	2000
EV00-3	1	Genesee, ID, USA	2000
SCH00-20	2	Walla Walla, WA, USA	2000
SCH00-22	1	Walla Walla, WA, USA	2000
SFL00-12	2	Pullman, WA, USA	2000
SPL00-41	2	Pullman, WA, USA	2000
SPL00-42	1	Pullman, WA, USA	2000
SPL00-55	1	Pullman, WA, USA	2000

[a]Mating type designation according to Barve *et al.* (2003).
[b]Isolates used in the characterization of chickpea differentials.
[c]Mating type not determined.
[d]Race designations were from Reddy & Kabbabeh (1985).
[e]Isolation date not available.

produce uniformly high relative humidity for 24 h to facilitate infection. Plants were then placed in a growth chamber (Conviron Model PGR15, Winnepeg, Manitoba, Canada) that was set at 12 h day (20°C) and 12 h night (16°C) at 100% relative humidity, or in a glasshouse. Control plants were sprayed with water, but otherwise were treated the same way as inoculated plants.

To assess reproducibility of the mini-dome bioassay, *D. rabiei* isolates AR19 and AR628 were used to inoculate cvs Dwelley and Spanish White in eight independent experiments over a 2-year period. Three experiments were conducted in the growth chamber and five experiments were conducted in a glasshouse.

Disease assessment

Two methods were used to assess disease severity 14 days after inoculation. The first method was based on the 1–9 rating scale, which was modified for seedling bioassays from Reddy & Singh (1984), as follows: 1, healthy plant, no disease; 2, lesions present, but small and inconspicuous; 3, lesions easily seen, but plant is mostly green; 4, severe lesions clearly visible; 5, lesions girdle stems, most leaves show lesions; 6, plant collapsing, tips die back; 7, plant dying, but at least three green leaves present; 8, nearly dead plant (virtually no green leaves left) but still with a green stem; and 9, dead plant (almost no green parts visible) (Fig. 1). In the second method, the number of leaves showing symptoms or wilting and the total number of leaves on each plant were counted and the percentage of infected leaves for each plant calculated. This leaf counting method provided a more objective and quantitative estimate of disease.

Thirty-four isolates of *D. rabiei* were used to inoculate cv. Dwelley, rated as moderately resistant to ascochyta blight. Two weeks after inoculation, disease severity was assessed using the 1–9 rating scale and then using percentage leaf infection. The two ratings were completed on the same day. There were six replicated pots, each with two plants for each isolate, and the experiment was performed twice in the Conviron growth chamber.

Characterization of chickpea differentials and commercial cultivars

Two experiments were conducted to evaluate the response of chickpea germplasm lines to inoculation with six isolates (A2-11 L, A3-2S, AR19, AR21, AR628 and CAB02-14). The first experiment included 40 germplasm lines (all listed in Table 1 except Billy Beans, Blanco Lechoso, Bronic, Burpee, CA9990I875 W, W6 2909, PI 552788 and PI 315818). The second experiment included eight germplasm lines that were not included in the first experiment plus three additional germplasm lines (Spanish White, Dwelley and PI 339363) that were included in the first experiment for comparison. Three pots (two plants each) were used for each treatment (each germplasm line by isolate combination). Each experiment was performed twice in the glasshouse where temperature was maintained between 16°C (night) and 22°C (day). Disease severity was assessed using the 1–9 rating scale only.

Comparison of pathotypes with previously identified races

Isolates AR19 and AR628 were directly compared with representative isolates of previously identified races, AR169 (race 3), AR650 (race 1), AR651 (race 2), AR652 (race 3), AR653 (race 4), AR654 (race 5) and AR655 (race 6). Chickpea genotypes used in this experiment included Spanish White, Dwelley, PI 559363 and five of the original six differentials, W6 22581 (ILC 249), W6 22584 (ILC 3279), W6 22585 (ILC 1591), W6 22587 (ILC 1903) and W6 22589 (ILC 3996), used by Reddy & Kabbabeh (1985). The experiment was performed three times, and disease severity was assessed using the 1–9 rating scale only.

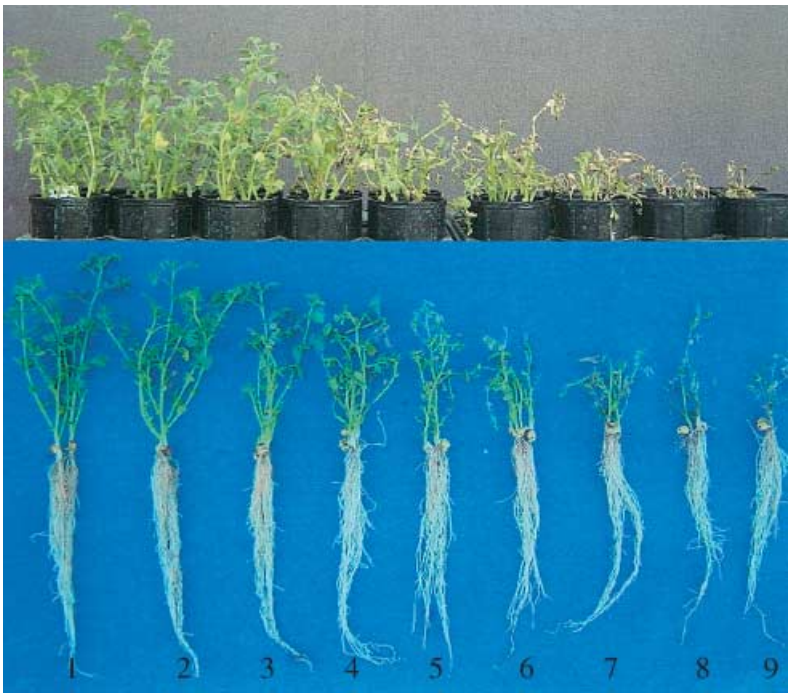

Figure 1 Illustration of the 1–9 disease rating scale for ascochyta blight of chickpea with the mini-dome bioassay 14 days after inoculation.

Field evaluations

Twelve chickpea cultivars, including two advanced breeding lines, were evaluated for resistance to ascochyta blight at the Spillman Experimental Farm of Washington State University near Pullman, Washington, in 2002 and 2003, and on a commercial farm near Genesee, Idaho, in 2003. Plots (3·6 × 2·5 m with 1·2 m fallow alley) with four replications were marked out in fields previously planted with chickpea and therefore containing abundant natural inoculum. No artificial inoculations were performed. Disease severity was rated twice during the growing season (9 July and 25 July 2002) using the 1–9 rating scale, and plots were hand-harvested to estimate yields.

Experimental design and data analyses

All glasshouse and growth chamber experiments were conducted using completely randomized designs. Disease severity scores were recorded for each plant and the average of the scores of the two plants in a pot represented one experimental unit. Bartlett's test of homogeneity of variance was used to determine if variance was independent of the mean. Analysis of variance was implemented using the program MINITAB (version 12, Minitab, Inc., State College, PA, USA), and Fisher's protected LSD at 0·01 significance level was used to compare treatment means. Residuals were plotted against observed values and predicted values to check for departure from normality. For correlation between the 1–9 rating scale and the percentage leaf infection, the data observations of individual experimental units (pots) were used. In assessing reproducibility of the mini-dome bioassay, data from the eight experiments were analysed separately using two-way ANOVA. The data were then combined and analysed using a three-way ANOVA (isolate, cultivar and block) where experiments were treated as blocks. The field plots were arranged in a randomized complete block design with four replications. Data were analysed using one-way ANOVA because the block effect was not significant in two-way ANOVA. Fisher's protected LSD at 0·05 significance level was used to compare cultivar means. For correlation between the mini-dome bioassay and field evaluations, the average disease scores of all six isolates on chickpea cultivars from the mini-dome bioassay were used to correlate with the disease severity scores of individual field plots of the same cultivars.

Results

Pathogenic variation of *D. rabiei* and correlation of the two disease assessment methods

The 34 isolates of *D. rabiei* exhibited significant variation in pathogenicity on cv. Dwelley. No evidence of unequal variance was found based on Bartlett's tests, and residuals showed a random distribution. The disease scores based on the 1–9 scale for individual isolates ranged from 1 to 8 (Fig. 2a), and the percentage leaf infection ranged from 10 to 100% (Fig. 2b). Significant differences in pathogenicity were detected among isolates (Fig. 2a and b). Isolates that caused high levels of disease severity and percentage leaf infection in the first experiment also caused high levels of disease in the second experiment. Some isolates, CAB01-7, CAB01-8, CAB02-2 and CAB02-5, showed some variation between experiments. Nevertheless, the disease scores from the two experiments were highly correlated (r = 0·97). The results obtained using the 1–9 rating scale and the results of percentage leaf infection were also highly correlated, r = 0·94 (Fig. 2c).

In the eight experiments designed to assess reproducibility of the mini-dome bioassay, a consistent pattern of disease severity caused by isolates AR19 and AR628 was observed on cvs Spanish White and Dwelley. Both isolates AR19 and AR628 caused high levels of disease severity (> 6) on Spanish White, but only isolate AR628 caused high levels of disease on Dwelley and isolate AR19 did not (data not shown). Three-factor ANOVA showed that all three factors (isolate, cultivar and experiment) and all their interactions were statistically significant (data not shown). The isolate factor accounted for 79% of the variation, followed by isolate–cultivar interaction (9%) and cultivar (5%). Although the absolute scores varied from experiment to experiment, the relative disease scores caused by the two isolates on cvs Spanish White and Dwelley were consistent. No differences were observed between the growth chamber experiments and the glasshouse experiments.

Characterization of chickpea differentials and commercial cultivars

Similar results were obtained in two independent experiments with 40 germplasm lines. When the germplasm lines were ranked by total disease score, the three most susceptible and the three most resistant lines were identical from both experiments. Some minor differences in ranking among chickpea lines with intermediate resistance were observed between the two experiments.

In the second experiment, the average disease scores of all 40 chickpea accessions infected with six isolates separated the isolates into two groups. Isolates AR19 and AR21 represented a low-pathogenicity group, whereas isolates A2-11 L, A3-2S, AR628, and CAB02-14 represented a high-pathogenicity group (Fig. 3). Further examination of the data from both experiments revealed that isolates of the low-pathogenicity group caused significant levels of disease (disease severity ratings ranged from 7 to 9) on only three chickpea accessions, PI 359075, W6-22584 and Spanish White, but only low levels of disease severity (scores less than 4) on the remaining 37 germplasm lines (Fig. 4a). The difference between resistant and susceptible responses to the low-pathogenicity isolates is clear with no intermediate interactions in the 40 chickpea lines tested (Fig. 4a). In contrast, the disease scores of the 40 chickpea germplasm lines caused by isolates of the high-pathogenicity group showed a continuous distribution ranging from 2 to 9 (Fig. 4b). The low-pathogenicity group is hereafter referred to as pathotype I, and the

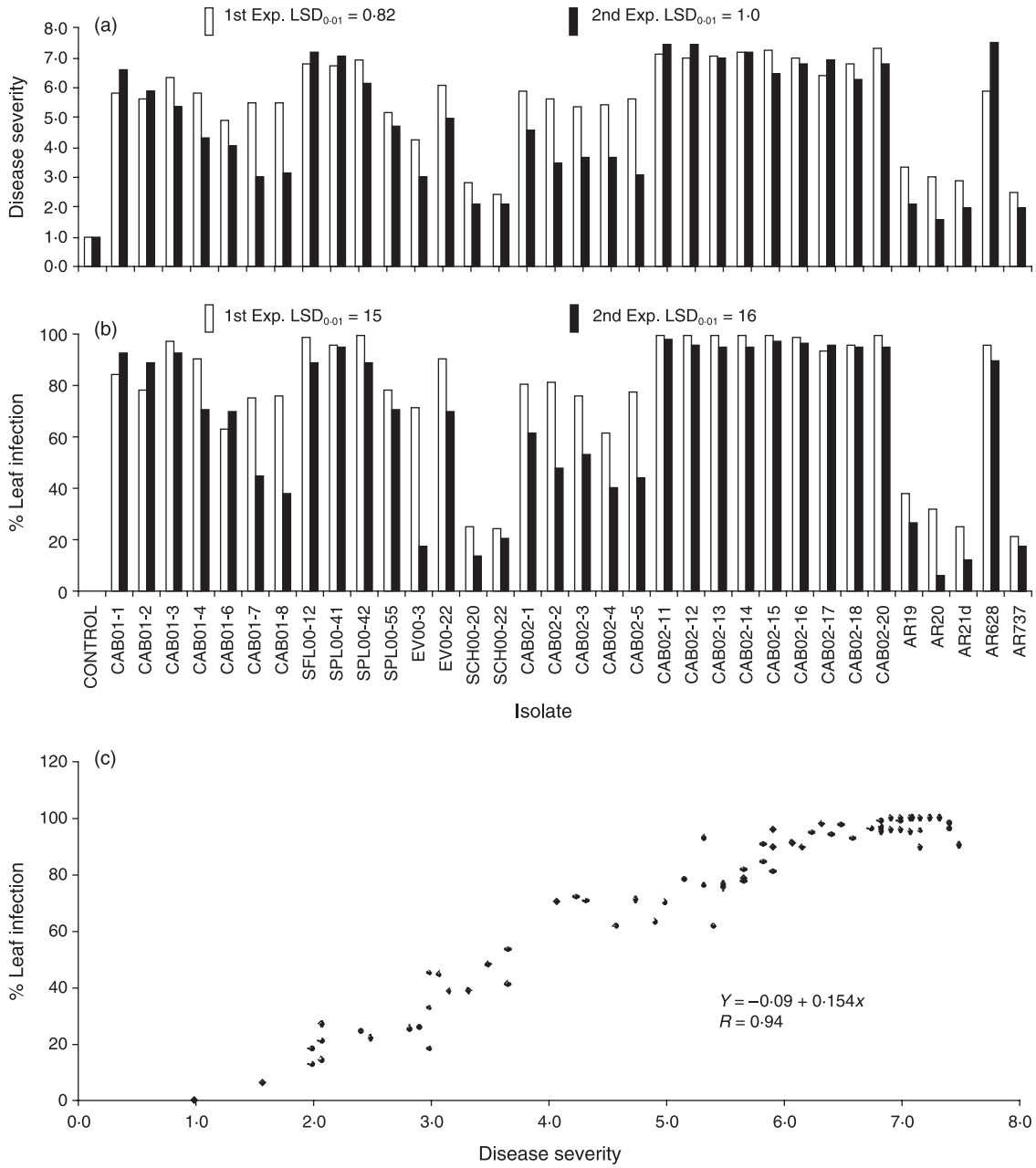

Figure 2 Mean disease severity (n = 6) of chickpea cv. Dwelley inoculated with 34 isolates of *Didymella rabiei* based on the 1–9 rating scale (a) and percentage leaf infection (b) in two separate experiments, and correlation of the two disease assessment methods (c).

high-pathogenicity group as pathotype II. Typical reactions of chickpea genotypes to inoculation with the two pathotypes are shown in Fig. 5. Spanish White was susceptible to both pathotypes; Dwelley was resistant to pathotype I but susceptible to pathotype II; and Sierra, a recent release of kabuli type cultivar, exhibited improved resistance to pathotype II in addition to pathotype I resistance. Chickpea (PI 559361), a desi type, showed good resistance to both pathotypes (Fig. 5). In the second experiment when eight germplasm lines were evaluated, cvs Burpee and Blanco Lechoso were susceptible to both pathotypes (disease scores > 7) and the other five genotypes were resistant to pathotype I, but showed various degrees of susceptibility to pathotype II (data not shown).

All chickpea germplasm lines examined in this study that were previously reported to be resistant to ascochyta blight were resistant to pathotype I. Only three germplasm lines (PI 559361, PI 559363 and W6 22589) showed high levels of resistance (disease scores < 4) to both pathotypes of *D. rabiei* found in the western US (Fig. 4). In addition, these accessions also showed excellent resistance in the

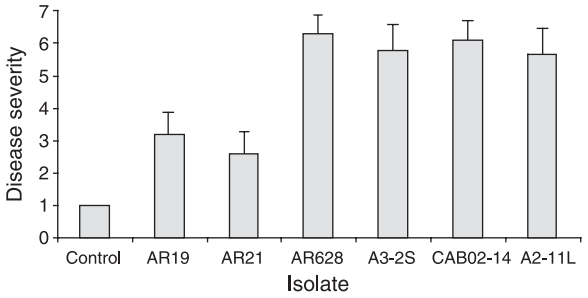

Figure 3 Mean disease severity of 40 chickpea genotypes caused by six isolates of *Didymella rabiei* (bar = standard deviation, $n = 120$). The isolates (AR19 and AR21) with low pathogenicity were referred to as pathotype I, and the isolates (A2-11 L, A3-2S, AR628 and CAB02-4) with high pathogenicity as pathotype II.

disease nursery at Pullman, WA, in 2002 and 2003 where both pathotypes of *D. rabiei* were present (FJM & WC, unpublished data).

Comparison of pathotypes with previously identified races

Results of the three pathogenicity trials used to directly compare pathotypes with the previously identified six races were generally in agreement, although the third showed lower overall levels of disease scores than the first two trials (Table 3). The representative isolates of races 1, 2, 3, 4 and 5 showed very similar pathogenicity patterns on the eight chickpea genotypes by severely affecting Spanish White and W6 22584, but causing low levels of disease on the other chickpea genotypes. This pathogenicity pattern closely resembled that of pathotype I isolate AR19 (Table 3). Among the isolates of the six races, isolate AR655 (race 6) was the most pathogenic, as shown by the high levels of disease severity on moderately resistant chickpea genotypes Dwelley, W6 22581, W6 22585 and W6 22587. The pathogenicity pattern of isolate AR655 was very similar to that of AR628 (pathotype II).

Field evaluations

Weather conditions in 2002 were conducive to ascochyta blight and disease was severe in the experimental plots. In contrast, near-record dry weather conditions occurred in 2003 and limited disease development. Data from 2002 showed that Myles was the most resistant cultivar followed by CDC-Yuma (Fig. 6). Spanish White, Surutato 77 and UC 27 were the most susceptible cultivars. The disease scores from the field plots on both assessment dates were highly correlated with disease scores obtained in the mini-dome bioassay in the glasshouse: $r = 0{\cdot}655$ ($P < 0{\cdot}001$) and $0{\cdot}614$ ($P < 0{\cdot}001$) for the first and second disease ratings, respectively. Comparison of yields with disease scores was not appropriate because cultivars have different yield potentials and are for different market classes. Nevertheless, cv. Myles produced the highest yields in the field, whereas susceptible cvs Spanish White, Surutato 77 and UC 27 produced few seeds.

Discussion

There are three findings from the present study that have important implications for future investigations on ascochyta blight of chickpea. First, most of the 22 differentials employed in previous studies reacted similarly to inoculations with each isolate of *D. rabiei*. Therefore the number of differentials can be reduced significantly without sacrificing accuracy in describing pathogenicity of the pathogen. Secondly, a two-pathotype system appears to be the best way to describe the current distribution of pathogenicty variation among isolates of *D. rabiei* from the western US. Thirdly, three chickpea germplasm lines were identified which are resistant to both pathotypes currently found in the US and can be employed in resistance breeding programmes.

Reducing the number of differentials required for identifying pathogenicity variation should facilitate future investigations to standardize the set of differentials in different chickpea production regions. Three differentials (W6 22584, W6 22582 and W6 22589) are recommended as a standard set for international comparisons, and each country or region may wish to add some local cultivars (such as Spanish White and Dwelley in the US) for relevance to local chickpea production.

The two pathotypes described here correspond to pathotypes I and II of the three-pathotype system of Udupa *et al.* (1998), but the third pathotype has not been

Table 3 Disease severity on eight chickpea differentials caused by representative isolates of two pathotypes (I and II) and six previously identified races of *Didymella rabiei*

Differential (Alternative identifier)	Check	AR19 (Pathotype I)	AR650 (Race 1)	AR651 (Race 2)	AR169 (Race 3)	AR652 (Race 3)	AR653 (Race 4)	AR654 (Race 5)	AR655 (Race 6)	AR628 (Pathotype II)
Spanish White	1·0	7·3	6·6	5·9	5·4	4·8	6·8	7·4	6·8	6·9
Dwelley	1·0	4·2	4·1	3·3	2·9	2·2	3·1	4·5	5·6	7·1
PI 559363 (ICC 12004)	1·0	2·4	1·2	1·7	1·6	1·2	1·8	1·7	2·3	2·3
W6 22584 (ILC 1292)	1·0	7·5	7·6	6·3	5·9	5·2	7·0	7·8	7·0	7·3
W6 22587 (ICC 1903)	1·0	3·4	2·7	1·8	1·6	1·5	3·5	3·2	4·5	4·7
W6 22581 (ILC 249)	1·0	3·3	2·0	2·0	1·9	1·3	3·2	2·4	5·9	5·8
W6 22585 (ILC 3279)	1·0	3·1	2·5	2·0	1·6	1·5	2·5	2·6	4·2	6·4
W6 22589 (ICC 3996)	1·0	1·9	1·3	1·2	1·2	1·0	2·2	1·5	2·9	1·8
					$LSD_{0\cdot01} = 1{\cdot}12$					

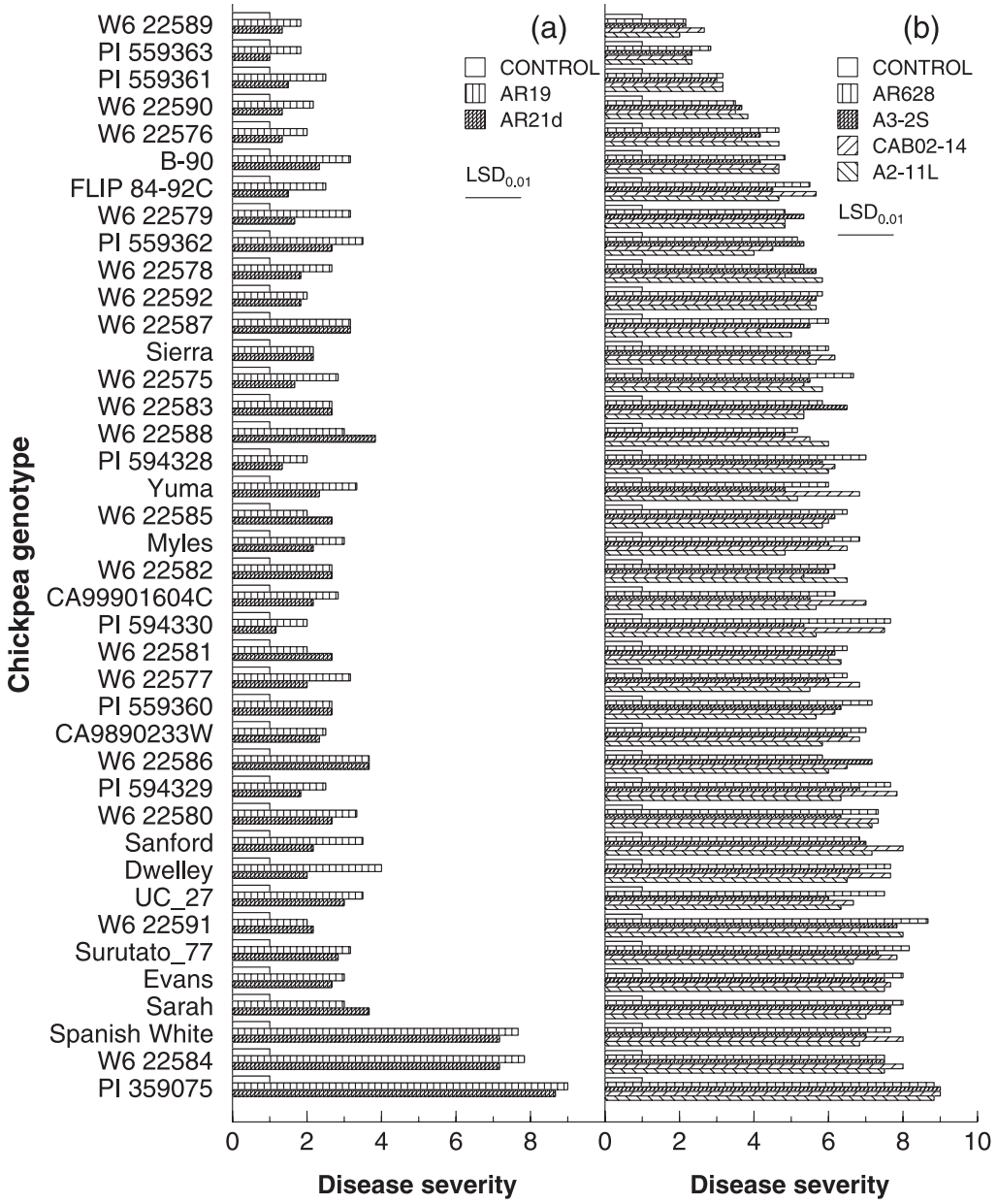

Figure 4 Disease severity of 40 chickpea genotypes after inoculation with isolates of pathotype I (a) and pathotype II (b) of *Didymella rabiei*. Each bar represents a mean of three replications.

found in the US. Udupa *et al.* (1998) used three differentials for three pathotypes: W6 22584 (ILC 1929), susceptible to all three pathotypes; W6 22582 (ILC 482), resistant to pathotype I, but susceptible to pathotypes II and III; and W6 22585 (ILC 3279), resistant to pathotypes I and II, but susceptible to pathotype III. However, the latter two differentials, W6 22582 and W6 22585, were both susceptible to pathotype II in the present study (Fig. 4b).

The six races of *D. rabiei* were originally defined based on six differentials with qualitative interaction without reversal of ranking (Reddy & Kabbabeh, 1985). However, there has been controversy in ranking the six races (Weising *et al.*, 1991). Results presented here (Table 3) showed that race 6 was the most pathogenic of the six and was the only race that showed high levels of pathogenicity on germplasm lines that are resistant to pathotype I. The other five races could not be reliably differentiated among themselves, supporting our hypothesis that race 6 is pathotype II and the other five races are pathotype I. Previous studies of pathogenicity of *D. rabiei* proposed 11–14 pathogenic groups, pathotypes, races or virulence forms based on resistant or susceptible reactions of different sets of differentials (Vir & Grewal, 1974; Singh, 1990; Jan & Wiese, 1991; Navas-Cortés *et al.*, 1998; Chongo *et al.*, 2004).

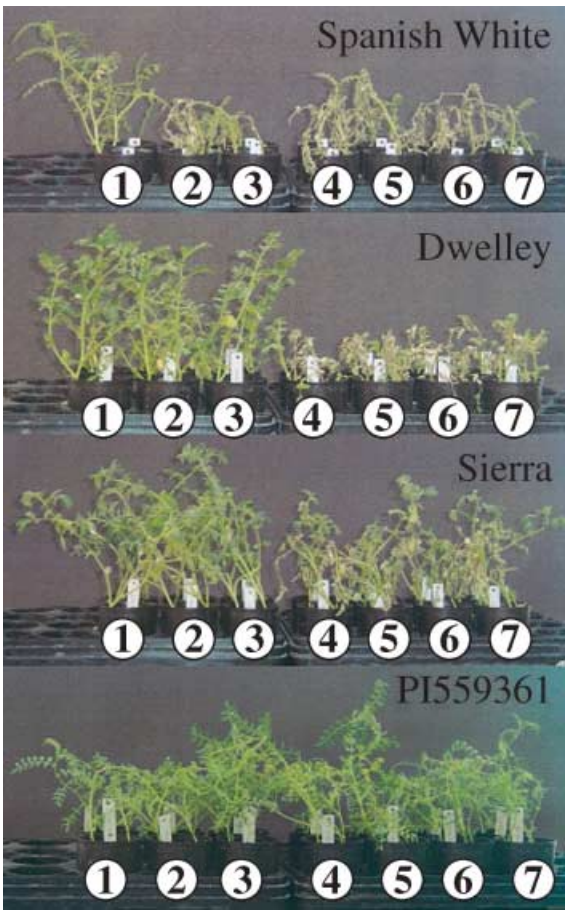

Figure 5 Disease phenotypes of four chickpea germplasm lines (Spanish White, Dwelley and Sierra, and PI 559361) after inoculation with six isolates of *Didymella rabiei*. 1, control; 2 and 3, isolates AR19 and AR21 of pathotype I; 4–6, isolates AR628, A3-2S, CAB02-14; and 7, A2-11 L of pathotype II.

Resistance or susceptibility was based on disease scores either above or below an arbitrary value (often 3, 4 or 5 on the 1–9 rating scale). With the known quantitative nature of this disease (Fig. 4b) and with the known environmental variability affecting pathogenicity assays (Fig. 1a), such arbitrary definitions of resistance and susceptibility are bound to generate inconsistent classifications.

Total disease scores caused by the six isolates on individual germplasm lines showed a continuous distribution, but when the disease responses were separated by pathotypes, different distribution patterns became evident (Fig. 4a and b). The disease severity levels caused by pathotype I isolates showed a bimodal distribution, suggesting a major gene conditioning the resistance, whereas the distribution of disease severity levels caused by pathotype II isolates was continuous, suggesting resistance to pathotype II may be multigenic. The distinct distribution patterns suggest that chickpea plants employ different resistance mechanisms (see Hamid & Strange, 2000) to the two pathotypes and that the two pathotypes may have different genetic mechanisms controlling pathogenicity. Isolates of *Didymella rabiei* produce phytotoxic solanapyrones (Alam *et al*., 1989; Höhl *et al*., 1991) and a proteinaceous phytotoxin (Chen & Strange, 1994) and perhaps phytotoxins may be virulence factors related to pathotypes.

Previous molecular studies of *D. rabiei* have found that neutral DNA markers were not correlated to virulence forms (Navas-Cortés *et al*., 1998; Udupa *et al*., 1998; Jamil *et al*., 2000; Santra *et al*., 2001). The result is not unexpected because the fungus appears to undergo frequent sexual reproduction and random mating (Peever *et al*., 2004). Establishment of a reliable and reproducible pathotyping procedure will help to identify molecular markers linked to pathotypes. Use of the two-pathotype system will not only simplify the pathotyping procedure while still capturing the major pathogenic variation of *D. rabiei* on chickpea, but will also facilitate future investigations into the mechanisms of chickpea resistance to ascochyta blight. Previous studies on the genetics of chickpea resistance used undefined isolates of *D. rabiei*, and resulted in different genetic hypotheses involving one, two or more resistance genes or quantitative trait loci (Hafiz & Ashraf, 1953; Singh & Reddy, 1983; Santra *et al*., 2000; Tekeoglu *et al*., 2000). Recent studies employing pathotype I or pathotype II isolates showed that resistance to pathotype I is conditioned by a single (major) gene, whereas resistance to pathotype II is conditioned by two or more independent loci (Udupa & Baum, 2003; Cho *et al*., 2004).

Disease control using host resistance is possible only if the resistance is effective against all pathotypes of the pathogen present in the region of crop cultivation (Porta-Puglia *et al*., 1994). Plant age is known to affect chickpea resistance to ascochyta blight, with older plants being more susceptible than younger plants (Chongo & Gossen, 2001). Therefore, resistance screening should relate to field performance to be useful for breeding programmes, as was the case in this study. Three chickpea accessions (PI 559361, PI 559363 and W6 22589) were identified to be resistant to both pathotypes I and II of *D. rabiei* found in the western US and these accessions may provide useful sources to be used in chickpea breeding programmes.

Acknowledgements

The authors thank Yung-Chun Chen for technical assistance with mini-dome bioassays, Rick Short for setting up field plots, Frank Dugan for providing the isolates of the previously described races of *D. rabiei*, Xianming Chen for discussions on statistical analyses of pathogenicity data, Timothy Paulitz for a presubmission review of the manuscript, and the USA Dry Pea and Lentil Council for partially funding this research.

References

Alam SS, Bilton JN, Slawin AMZ, Williams DJ, Sheppard RN, Strange RN, 1989. Chickpea blight: production of the phytotoxins solanapyrones A and C by *Ascochyta rabiei*. *Phytochemistry* **28**, 2627–30.

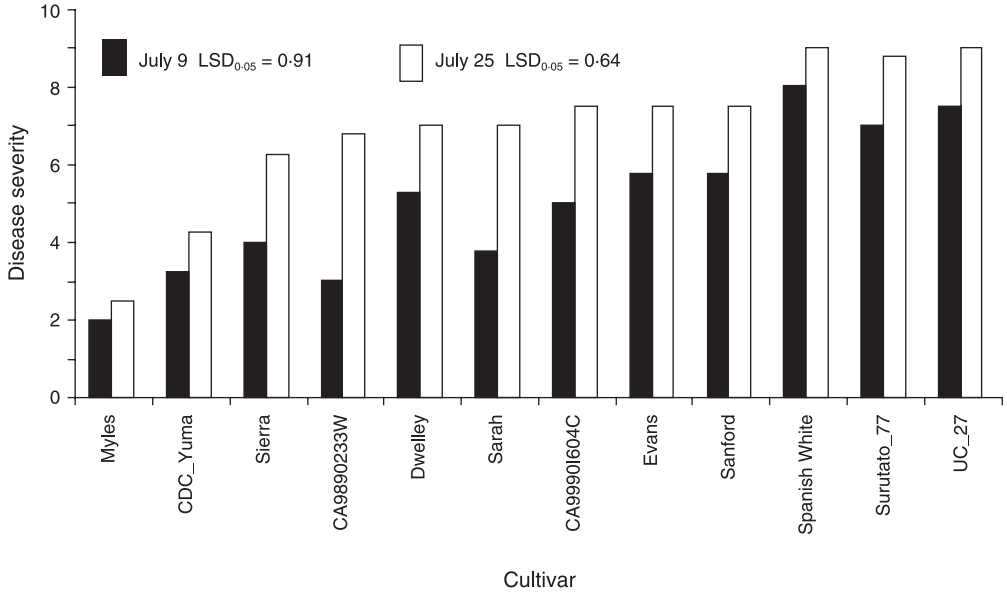

Figure 6 Severity of ascochyta blight on 12 cultivars and advanced breeding lines in field plots on two disease rating dates. Each bar represents a mean of four replications.

Barve MP, Arie T, Salimath SS, Muehlbauer FJ, Peever TL, 2003. Cloning and characterization of the mating type (*MAT*) locus from *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) and a *MAT* phylogeny of legume-associated *Ascochyta* spp. *Fungal Genetics and Biology* **39**, 151–67.

Chen W, Muehlbauer FJ, 2003. An improved technique for virulence assay of *Ascochyta rabiei* on chickpea. *International Chickpea and Pigeonpea Newsletter* **10**, 31–3.

Chen YM, Strange RN, 1994. Production of a proteinaceous phytotoxin by *Ascochyta rabiei* grown in expressed chickpea sap. *Plant Pathology* **43**, 321–7.

Cho S, Chen W, Muehlbauer FJ, 2004. Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. *Theoretical and Applied Genetics* **109**, 733–9.

Chongo G, Gossen BD, 2001. Effect of plant age on resistance to *Ascochyta rabiei* in chickpea. *Canadian Journal of Plant Pathology* **23**, 358–63.

Chongo G, Gossen BD, Buchwaldt L, Adhikari T, Rimmer SR, 2004. Genetic diversity of *Ascochyta rabiei* in Canada. *Plant Disease* **88**, 4–10.

Geistlinger J, Weising K, Kaiser WJ, Kahl G, 1997. Allelic variation at a hypervariable compound microsatellite locus in the ascomycete *Ascochyta rabiei*. *Molecular and General Genetics* **256**, 298–305.

Gowen SR, Orton M, Thurley B, White A, 1989. Variation in pathogenicity of *Ascochyta rabiei* on chickpeas. *Tropical Pest Management* **35**, 180–6.

Hafiz A, Ashraf M, 1953. Studies on inheritance of resistance to Mycosphaerella blight in gram. *Phytopathology* **43**, 580–1.

Hamid K, Strange RN, 2000. Phytotoxicity of solanapyrones A and B produced by the chickpea pathogen *Ascochyta rabiei* (Pass.) Labr. & the apparent metabolism of solanapyrone A by chickpea tissues. *Physiological and Molecular Plant Pathology* **56**, 235–44.

Höhl B, Weidemann C, Höhl C, Barz W, 1991. Isolation of solanopyrones A, B and C from culture filtrates of *Ascochyta rabiei* and aspects of phytotoxin action. *Journal of Phytopathology* **132**, 193–6.

Jamil FF, Sarwar N, Sarwar M, Khan JA, Geistlinger J, Kahl G, 2000. Genetic and pathological diversity within *Ascochyta rabiei* (Pass.) laboratory populations in Pakistan causing blight of chickpea (*Cicer arietinum* L.). *Physiological and Molecular Plant Pathology* **57**, 243–54.

Jan H, Wiese MV, 1991. Virulence forms of *Ascochyta rabiei* affecting chickpea in the Palouse. *Plant Disease* **75**, 904–6.

Kaiser WJ, Kusmenoglu I, 1997. Distribution of mating types and the teleomorph of *Ascochyta rabiei* on chickpea in Turkey. *Plant Disease* **81**, 1284–7.

Lichtenzveig J, Shtienberg D, Zhang HB, Bonfil DJ, Abbo S, 2002. Biometric analyses of the inheritance of resistance to *Didymella rabiei* in chickpea. *Phytopathology* **92**, 417–23.

Muehlbauer FJ, Kaiser WJ, 1994. Using host resistance to manage biotic stresses in cool season food legumes. *Euphytica* **73**, 1–10.

Muehlbauer FJ, Kaiser WJ, Kusmenoglu I, 1998a. Registration of 'Sanford' chickpea. *Crop Science* **38**, 282.

Muehlbauer FJ, Kaiser WJ, Kusmenoglu I, 1998b. Registration of 'Dwelley' chickpea. *Crop Science* **38**, 283.

Navas-Cortés JA, Perez-Artes E, Jimenez-Diaz RM, Llobell A, Bainbridge BW, Heale JB, 1998. Mating type, pathotype and RAPD analysis in *Didymella rabiei*, the agent of Ascochyta blight of chickpea. *Phytoparasitica* **26**, 199–212.

Nene YL, 1982. A review of Ascochyta blight of chickpea. *Tropical Pest Management* **28**, 61–70.

Nene YL, Reddy MV, 1987. Chickpea diseases and their control. In: Sexena MC, Singh KB, eds. *The Chickpea*. Wallingford, UK: CAB International, 233–370.

Peever TL, Salimath SS, Su G, Kaiser WJ, Muehlbauer FJ, 2004. Historical and contemporary multilocus population structure of *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) in the

Pacific Northwest of the United States. *Molecular Ecology* **13**, 291–309.
Porta-Puglia A, Bernier CC, Jellis GJ, Kaiser WJ, Reddy MV, 1994. Screening techniques and sources of resistance to foliar diseases caused by fungi and bacteria in cool season food legumes. *Euphytica* **73**, 11–25.
Porta-Puglia A, Crino P, Mosconi C, 1996. Variability in virulence to chickpea of an Italian population of *Ascochyta rabiei*. *Plant Disease* **80**, 39–41.
Reddy MV, Kabbabeh S, 1985. Pathogenic variability in *Ascochyta rabiei* (Pass.) Lab. in Syria and Lebanon. *Phytopathologia Mediterranea* **24**, 265–6.
Reddy MV, Singh KB, 1984. Evaluation of a world collection of chickpea germplasm accessions for resistance to Ascochyta blight. *Plant Disease* **68**, 900–1.
Reddy MV, Singh KB, 1992. Registration of five chickpea germplasm lines resistant to Ascochyta blight. *Crop Science* **32**, 1079–80.
Riahi H, Harrabi MM, Halila MH, Strange RE, 1990. A quantitative scale for assessing chickpea reaction to *Ascochyta rabiei*. *Canadian Journal of Botany* **68**, 2736–8.
Santra DK, Singh G, Kaiser WJ, Gupta VS, Ranjekar PK, Muehlbauer FJ, 2001. Molecular analysis of *Ascochyta rabiei* (Pass.) Labr., the pathogen of Ascochyta blight in chickpea. *Theoretical and Applied Genetics* **102**, 676–82.
Santra DK, Tekeoglu M, Ratnaparkhe M-L, Kaiser WJ, Muehlbauer FJ, 2000. Identification and mapping of QTLs conferring resistance to Ascochyta blight in chickpea. *Crop Science* **40**, 1606–12.
Singh G, 1990. Identification and designation of physiological races of *Ascochyta rabiei* in India. *Indian Phytopathology* **43**, 48–52.
Singh KB, Malhotra RS, Saxena MC, 1997. Registration of FLIP 91-178C, FLIP 93-53C, and FLIP 93-98C: chickpea germplasm lines resistant to Ascochyta blight, Fusarium wilt and cold. *Crop Science* **37**, 633.
Singh KB, Reddy MV, 1983. Inheritance of resistance to Ascochyta blight in chickpea. *Crop Science* **23**, 9–10.
Singh KB, Reddy MV, Nene YL, 1984. International testing of chickpeas for resistance to Ascochyta blight. *Plant Disease* **68**, 782–4.
Tekeoglu M, Santra DK, Kaiser WJ, Muehlbauer FJ, 2000. Ascochyta blight resistance inheritance in three chickpea recombinant inbred line populations. *Crop Science* **40**, 1251–6.
Trapero-Casas A, Kaiser WJ, 1992a. Influence of temperature, wetness period, plant age, and inoculum concentration on infection and development of Ascochyta blight of chickpea. *Phytopathology* **82**, 589–96.
Trapero-Casas A, Kaiser WJ, 1992b. Development of *Didymella rabiei*, the teleomorph of *Ascochyta rabiei*, on chickpea straw. *Phytopathology* **82**, 1261–6.
Trapero-Casas A, Navas-Cortés JA, Jimenez-Diaz RM, 1996. Airborne ascospores of *Didymella rabiei* as a major primary inoculum for Ascochyta blight epidemics in chickpea crops in southern Spain. *European Journal of Plant Pathology* **102**, 237–45.
Udupa SM, Baum M, 2003. Genetic dissection of pathotype-specific resistance to Ascochyta blight disease in chickpea (*Cicer arietinum* L.) using microsatellite markers. *Theoretical and Applied Genetics* **106**, 1196–202.
Udupa SM, Weigand F, Saxena MC, Kahl G, 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the Ascochyta blight pathogen of chickpea. *Theoretical and Applied Genetics* **97**, 299–307.
Vir S, Grewal JS, 1974. Physiologic specialization in *Ascochyta rabiei* the causal organism of gram blight. *Indian Phytopathology* **27**, 355–60.
Weising K, Kaemmer D, Epplen JT, Weigand F, Saxena M, Kahl G, 1991. DNA fingerprinting of *Ascochyta rabiei* with synthetic oligodeoxynucleotides. *Current Genetics* **19**, 483–9.